IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: David James CRAIK Norelle Lee DALY Katherine Justine NIELSEN	Attn: Office of Petitions
Serial No.: Unknown	Related to: International Application No. PCT/AU99/00769
Filed: Between 14 May 2000 and 14 March 2001	Filed: 14 September 1999
For: CYCLISED CONOTOXIN PEPTIDES	Priority: Australian Application No. PP 5895 filed 14 September 1998

PROTEST UNDER 37 C.F.R. § 1.291(a)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The protestor through the undersigned respectfully requests that the materials enclosed herewith be considered during examination of the above-identified patent application ("the Craik U.S. application').

1. Tam, J.P. and Y.A. Lu (1997). "Synthesis of large cyclic cysteine-knot peptide by orthogonal coupling strategy using unprotected peptide precursors." Tetrahedron Letters **38**: 5599-5602.

2. Tam, J.P. and Y.A. Lu (1998). "A biomimetic strategy in the synthesis and fragmentation of cyclic protein." Protein Sci **7**: 1583-92.

3. Zhang, L. and J. P. Tam (1997). "Synthesis and Application of Unprotected Cyclic Peptides as Building Blocks for Peptide Dendrimers." J Am Chem Soc **119**(10): 2363-70.

4. Hruby, V.J. (1982). "Conformational restrictions of biologically active peptides via amino acid side chain groups." Life Sci **31**(3): 189-99.

5. Okumu, F. W., G. M. Pauletti, et al. (1997). "Effect of restricted conformational flexibility on the permeation of model hexapeptides across Caco-2 cell monolayers." Pharm Res **14**(2): 169-75.

6. Terada, S., T. Kato, et al. (1975). "Synthesis and hydrolysis by pepsin and trypsin of a cyclic hexapeptide containing lysine and phenylalanine." Eur J Biochem **52**(2): 273-82.

7. Al-Obeidi, F., A. M. Castrucci, et al. (1989). "Potent and prolonged acting cyclic lactam analogues of alpha-melanotropin: design based on molecular dynamics." J Med Chem **32**(12): 2555-61.

8. Charpentier, B., A. Dor, et al. (1989). "Synthesis and binding affinities of cyclic and related linear analogues of CCK8 selective for central receptors." J Med Chem **32**(6): 1184-90.

9. Claeson, P., U. Goransson, et al. (1998). "Fractionation Protocol for the Isolation of Polypeptides from Plant Biomass." J Nat Prod **61**(1): 77-81.

10. Saether, O., D. J. Craik, et al. (1995). "Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1." Biochemistry **34**(13): 4147-58.

11. Pallaghy, P.K., K.J. Nielsen, et al. (1994). "A common structural motif incorporating a cystine knot and a triple-stranded beta-sheet in toxic and inhibitory polypeptides." Protein Sci **3**: 1833-9.

12. Goldenberg, D. P. and T. E. Creighton (1983). "Circular and circularly permuted forms of bovine pancreatic trypsin inhibitor." J Mol Biol **165**(2): 407-13.

13. Goldenberg, D. P. and T. E. Creighton (1984). "Folding pathway of a circular form of bovine pancreatic trypsin inhibitor." J Mol Biol **179**(3): 527-45.

14. Armishaw, C. J., Dutton, J. Hogg, R.C., Adams, D.J., Craik, D.J., Alewood, P.F. (2001). Synthesis of N to C Terminal Cyclic Analogues of alpha-Conotoxin ImI by Chemoselective Ligation of Unprotected Linear Precursors. Peptides: The Wave of the Future. R. A. H. Michael Lebl, American Peptide Society**:** 113-114.

I. DISCUSSION OF THE CITED REFERENCES

The protestor has not reviewed the claims in the Craik U.S. application[1] and requests that all pending claims in the application be examined in view of these references. To the extent that the pending claims in the Craik U.S. application are similar to the claims in the Craik PCT application, these references may, either alone or in combination, render one or more of the pending claims unpatentable. To facilitate review of these references, the following discussion highlights the particular relevance of these references.

1. Tam, J.P. and Y.A. Lu (1997). "Synthesis of large cyclic cysteine-knot peptide by orthogonal coupling strategy using unprotected peptide precursors." Tetrahedron Letters **38**: 5599-5602.

Tam and Lu describes the chemistry utilized for N to C cyclization used and claimed in the Craik U.S. application. See particularly Fig. 2 showing the reaction scheme and the text starting at the bottom of page 5599 through page 5602.

2. Tam, J.P. and Y.A. Lu (1998). "A biomimetic strategy in the synthesis and fragmentation of cyclic protein." Protein Sci **7**: 1583-92.

The abstract on page 1583, Figure 2 on page 1584 and the text above it (1st paragraph), the Synthesis of Linear Precursor section on page 1585, and the Thia Zip Cyclization section on pages 1585-6 all illustrate the first method of cyclizing a peptide as illustrated on page 5-6 of the Craik U.S. application (particularly relevant to claims 11-13, but also to cyclized and cyclic composition claims 1-10). This publication shows that the peptide is built onto the resin using an S-CH_2-CH_2-CO linker (the Synthesis of Linear Precursor section on page 1585, middle of the paragraph). This is the same linker used in Example 1 of the Craik U.S. application. On page 1586, the two-step method to form disulfide bonds section discusses drawbacks of using one-step oxidation (folding) of peptides (particularly cyclized peptides) and states that improperly folded forms are common. This is relevant to Examples 1 and 2 of the Craik U.S. application wherein

[1] Protestor believes that a U.S. patent application was filed under 35 U.S.C. § 371 from international application number PCT/AU99/00769 filed 14 September 1999 claiming priority to Australian application number PP 5895 filed 14 September 1998.

one-step folding is used. No determination of cysteine pattern is performed in the Craik U.S. application. In fact, following the method of Example 2 of the Craik U.S. application results in multiple folding forms after the reaction.

3. Zhang, L. and J. P. Tam (1997). "Synthesis and Application of Unprotected Cyclic Peptides as Building Blocks for Peptide Dendrimers." J Am Chem Soc **119**(10): 2363-70.

Zhang, et al. describe and discuss the chemistry employed in the cyclization method of the Craik U.S. application. Figure 1 on page 2364 shows the same chemistry as in Article 1 above and in the Craik U.S. application. The fact that cysteines could be oxidized into disulfide bonds (one pair of cysteines to form one disulfide bond) is demonstrated in Figure 6 and in the second paragraph of the second column on page 2367, beginning, "To determine whether the internal cysteine...". Of particular note is the discussion concerning the production of a bicyclic peptide (having N to C cyclized backbone and a disulfide bond) (Subsection 1 under "Applications" paragraph). Page 2363, second column, second paragraph (beginning "Cyclic peptides have several advantages...") discusses the advantages of cyclized peptides, as set out in the Craik U.S. application, namely metabolic stability and receptor selectivity, as being general concepts in peptide chemistry. This includes all bioactive peptides of which conotoxins are a part. Thus, this publication is relevant to the advantages stipulated in the Craik U.S. application and to the alleged invention as claimed in the Craik U.S. application.

4. Hruby, V.J. (1982). "Conformational restrictions of biologically active peptides via amino acid side chain groups." Life Sci **31**(3): 189-99.

Hruby states that determination of the relationship between conformation and biological activity for peptide hormones is difficult due to the conformational flexibility of these peptides in solution. His approach to overcome this difficulty was to cyclize these peptides through covalent bonding of side chain groups of residues in the peptide. Biologically active peptides that had been cyclized were found to have: 1) greater conformational integrity; 2) increased agonist or antagonist potency; 3) prolonged biological activity; 4) increased enzymatic stability and 5) increased specificity for a particular receptor. This article demonstrates that the idea of adding

structural constraints to a peptide in order to increase stability, activity, selectivity, etc. is not new.

5. Okumu, F. W., G. M. Pauletti, et al. (1997). "Effect of restricted conformational flexibility on the permeation of model hexapeptides across Caco-2 cell monolayers." Pharm Res **14**(2): 169-75.

Okumu, et al. compared the cellular permeation characteristics of synthesized linear and cyclic hexapeptides using the Caco-2 cell culture model. The results showed that the cyclic hexapeptides were 2 to 3 times better able to permeate the Caco-2 cell monolayer than were the linear hexapeptides. From these results, it was determined that cyclization of the linear hexapeptides increased their lipophilicity, thus enhancing their potential bioavailability. Increasing bioavailability through cyclization of peptides, therefore, has been established in the literature before the Craik U.S. application's priority date.

6. Terada, S., T. Kato, et al. (1975). "Synthesis and hydrolysis by pepsin and trypsin of a cyclic hexapeptide containing lysine and phenylalanine." Eur J Biochem **52**(2): 273-82.

Terada, et al. demonstrate that cyclic hexapeptides are much more resistant to hydrolysis by peptidases than are their linear equivalents. Results showed that cyclo(-Gly_2-Phe_2-Gly-Lys) was not hydrolyzed at all by trypsin and was hydrolyzed 10,000 times more slowly than the linear hexapeptide Phe-Gly-Lys-Gly_2-Phe. This article, as well as Article 2 above, demonstrates that cyclization of a peptide can lead to increased resistance to proteolysis by peptidases.

7. Al-Obeidi, F., A. M. Castrucci, et al. (1989). "Potent and prolonged acting cyclic lactam analogues of alpha-melanotropin: design based on molecular dynamics." J Med Chem **32**(12): 2555-61.

In these experiments, Al-Obeidi, et al. designed a new class of cyclic α-melanotropin analogs through lactam bridge formation between side chain groups. One of the goals was to design an analog of α-melanotropin with high potency and prolonged duration of action. Results showed that certain cyclic α-melanotropins exhibited 100-fold higher potency *in vitro* and a

significant increase in duration of action in the frog and lizard skin assay compared to α-melanotropin. Increased potency and prolonged biological activity through cyclization of peptides was clearly demonstrated well before the Craik U.S. application's priority date.

8. Charpentier, B., A. Dor, et al. (1989). "Synthesis and binding affinities of cyclic and related linear analogues of CCK8 selective for central receptors." J Med Chem **32**(6): 1184-90.

Charpentier, et al. demonstrate that cyclic analogs of cholecystokinin (CCK_8) display higher affinities for central-type binding sites than the linear analogs of CCK_8. The selectivity of these peptides were measured by their ability to displace [^{3}H]propionyl-CCK_8 from guinea pig brain and pancreatic membranes. A certain series of cyclic CCK8 analogs were shown to have significantly increased affinity for the central (brain) binding sites, while the affinity for pancreatic receptors were not affected. This article demonstrates that increased receptor subtype selectivity through peptide cyclization was known prior to the Craik U.S. application.

9. Claeson, P., U. Goransson, et al. (1998). "Fractionation Protocol for the Isolation of Polypeptides from Plant Biomass." J Nat Prod **61**(1): 77-81.

This publication contains a description of the isolation and sequencing of a cyclic peptide from a plant. The peptide, named varv peptide A, contains the amino acid sequence "TRNGLPV" (see last line of the abstract on page 77 and top of second column on page 79). This has relevance to the claimed linker sequences in the Craik U.S. application.

10. Saether, O., D. J. Craik, et al. (1995). "Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1." Biochemistry **34**(13): 4147-58.

The same linker sequence as discussed above in Claeson, et al. is also found in the plant peptide kalata B1, which is shown to have a cyclic polypeptide backbone (see Figure 8, page 4155).

11. Pallaghy, P.K., K.J. Nielsen, et al. (1994). "A common structural motif incorporating a cystine knot and a triple-stranded beta-sheet in toxic and inhibitory polypeptides." Protein Sci **3**: 1833-9.

The abstract on page 1833 discusses the strong structural similarities between a conotoxin (ω-conotoxin GIVA) and the cyclic peptide, kalata B1, and the class of peptides known as trypsin inhibitors. This is shown in Figures 1-3 on pages 1834-5. It is further discussed in the text on page 1834, second column, second paragraph continuing through to the second column of page 1835. This is especially relevant to cyclization of BPTI (a member of the trypsin inhibitor class that contains the inhibitor cysteine knot motif) which is discussed in Article 11 below. The first paragraph of the conclusion, page 1838, discusses the extreme stability that the cysteine knot lends to all peptides containing it without regard to whether the peptide is cyclized (kalata) or not (ω-conotoxin GIVA).

12. Goldenberg, D. P. and T. E. Creighton (1983). "Circular and circularly permuted forms of bovine pancreatic trypsin inhibitor." J Mol Biol **165**(2): 407-13.

This publication describes the cyclization of BPTI (a member of the trypsin inhibitor family). Page 406, second paragraph discusses the cyclization and page 412 discusses the retention of activity. This publication is relevant to claims 1-19 of the Craik U.S. application.

Articles 11 and 12, taken together, are particularly relevant to the Craik U.S. application. They teach the strong structural similarities of each member of the inhibitor cysteine knot (ICK) peptides (of which ω-conotoxins, trypsin inhibitors and kalata B1 are all members). Further, these references teach that non-cyclized ICK peptides may be cyclized without loss of activity. Finally, these references also teach that the ICK motif lends extreme structural stability to the molecule, thus making it likely that additional benefits will not be gained by cyclization.

13. Goldenberg, D. P. and T. E. Creighton (1984). "Folding pathway of a circular form of bovine pancreatic trypsin inhibitor." J Mol Biol **179**(3): 527-45.

In order to study the roles of the N and C terminal regions of bovine pancreatic trypsin inhibitor (BPTI), Goldenberg and Creighton prepared a modified form of BPTI in which the N-terminus amino and C-terminus carboxyl groups were linked together in a peptide bond, creating a circular backbone. The expected result from the linked termini was an overall stabilization of the native folded conformational state. The native conformation was not measurably stabilized by the cross-link, however, indicating that the native state of the circular protein had a slightly strained conformation. This publication teaches that cyclization may reduce the entropy of the folded state or introduce steric strain, and thus actually destabilize the folded state of the protein. Thus, the benefits of cyclization are unpredictable.

14. Armishaw, C. J., Dutton, J. Hogg, R.C., Adams, D.J., Craik, D.J., Alewood, P.F. (2001). Synthesis of N to C Terminal Cyclic Analogues of alpha-Conotoxin ImI by Chemoselective Ligation of Unprotected Linear Precursors. Peptides: The Wave of the Future. R. A. H. Michael Lebl, American Peptide Society: 113-114.

Armishaw, et al. illustrate how difficult and unpredictable cyclization of conopeptides may be. Despite having the three-dimensional structure of ImI, knowledge of the close proximity of the termini, knowledge of the position of the active site residues and having extensive experience in cyclizing peptides, the author's efforts to cyclize α-conotoxin ImI resulted in a 30-fold decrease in receptor binding activity, instead of enhanced biological activity, as is predicted and claimed in the Craik U.S. application. The difficulty of cyclization is unequivocally confirmed by applicant Craik himself who attempted but failed to cyclize α-conotoxin ImI utilizing the methods claimed in the Craik U.S. application.

II. CONCLUSION

The protestor has not reviewed the claims in the Craik U.S. application and requests that all pending claims in the application be examined in view of these references. To the extent that the pending claims in the Craik U.S. application are similar to the claims in the Craik PCT

application, these references may, either alone or in combination, render one or more of the pending claims unpatentable.

Additionally, the protestor hereby certifies that a duplicate copy of this Protest and all cited references has been sent to The University of Queensland, the applicant named in the international application, and to Michael James Caine, the agent named in the international application, by first class, airmail on 14 April 2003, at the following addresses:

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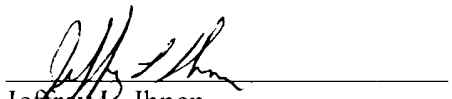
Respectfully submitted,



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